

CURCUMIN AND CURCUMINOID INHIBITION OF ANGIOGENESIS

Background of the Invention

The invention is generally in the field of methods of inhibiting
5 angiogenesis, and more specifically is drawn to methods and compositions for inhibiting angiogenesis.

The United States government has rights in this invention by virtue of grant R03 AR44947 from the National Institutes of Health.

Current treatments of cancer and related diseases have limited
10 effectiveness and numerous serious unintended effects. Based primarily on chemical, radiation and surgical therapy, these treatments have progressed only incrementally during more than thirty years of intensive research to discover the origins and devise improved therapies of neoplastic diseases.

Current research strategies emphasize the search for effective therapeutic
15 modes with less risk, including the use of natural products and biological agents. This change in emphasis has been stimulated by the fact that many of the consequences, to patients and their offspring, of conventional cancer treatment, including new cancers, mutations and congenital defects, result from their actions on genetic material and mechanisms. Hong et al., J. Natl. Cancer Inst.
20 Monogr. 17:49-53 (1995). Efforts continue to discover the origins of cancer at the genetic level, and correspondingly new treatments, but such interventions also may have serious unanticipated effects.

The observation by Folkman that tumors are highly vascular, and the elucidation by him and others of a process termed *angiogenesis* through which
25 many tumors derive a blood supply by the generation of microvessels, provided an important new avenue to therapy of cancer and other diseases and disorders. Folkman, Proc. Natl. Acad. Sci. U. S. A. 95(16):9064-6 (1998); C. R. Acad. Sci. III 316(9):909-918 (1993). Angiogenesis has now been recognized in inflammatory lesions and benign tumors, in addition to malignant tumors.

30 Mammals are characterized by complex cardiovascular systems that enable their warm-blooded nature, internal embryonic and fetal development and

successful population of extreme habitats. The development of an extensive capillary system, specialized in each organ and tissue, is an essential feature of mammalian cardiovascular systems, to provide optimal distribution of nutrients and other substances including hormones and defensive agents. The metabolic and physiologic needs of mammalian cells are met by their proximity to capillaries, and limited resources may be diverted by imbalance of this supply system. Tortora, "Principles of Human Anatomy", 5th ed., pp. 371-372, Harper & Row, N. Y. (1989).

Angiogenesis results primarily from the development of new or lengthened capillaries, and larger microvessels. Capillaries are formed primarily of specialized endothelial cells and the connective tissue layer to which they adhere, the basement membrane. The proliferation of endothelial cells and their migration and orientation to form capillaries is recognized as the key process regulated in the control of angiogenesis. Neovascularization is a form of angiogenesis marked by formation of blood vessels in a tissue or region previously devoid of blood vessel supply, for example the cornea of the eye. The mechanisms involved in angiogenesis are quite complicated, however, and no one appears to be the sole controlling mechanism.

Mammals have effective mechanisms to regulate this vital process. Stimulation of angiogenesis in adult mammals, other than as a part of normal tissue repair, pregnancy or the menstrual cycle, is abnormal and often pathological. Many malignant tumors, benign tumors and inflammatory lesions have the ability to evade or mobilize these regulatory mechanisms to support their growth and further malignant progression.

Development of effective preventive and treatment means has been hampered by inadequate understanding of the factors controlling this process. The premise of therapeutic development for such conditions is that effective treatment does not require destruction of the cells or tissues of origin. Reduction or prevention of the increased blood supply can be sufficient to prevent their

growth, and the manifestation of the condition as a disease or pathological disorder.

This concept was initially rejected, but widespread recognition of angiogenesis as a major factor in a variety of pathological conditions and diseases, particularly cancer and pre-cancerous conditions, has occurred recently among scientists and businesses. It is estimated that 184 million U. S. and European Union (EU) disease cases could benefit from treatment to inhibit angiogenesis that is inappropriate and pathological (anti-angiogenic therapy), in addition to an estimated 314 million disease cases in the U.S. and EU that might benefit from treatment to stimulate angiogenesis, for example in cardiac rehabilitation. Thirty-one specific projects of pharmaceutical and biotechnology companies to develop anti-angiogenic treatment were reported in Gen. Eng. News 18(17):1, 8, 34, 46 (1998).

It is an object of the present invention to provide methods of treating a mammal having a disease or condition characterized by increased angiogenesis.

It is a further object of the present invention to provide a method of preventing the initiation or progression of a disease or condition characterized by increased angiogenesis in a mammal, especially skin diseases and diseases characterized by elevated basic fibroblast growth factor.

Summary of the Invention

Methods for treating diseases or disorders of the skin which are characterized by angiogenesis have been developed using curcumin and curcumin analogs. Based on the results obtained with curcumin, it has been determined that other angiogenesis inhibitors can also be used to treat these skin disorders. It was also discovered that curcumin acts to inhibit angiogenesis in part by inhibition of basic fibroblast growth factor (bFGF), and thereby provides a means for treating other disorders characterized by elevated levels of bFGF, such as bladder cancer, using curcumin and other analogues which also inhibit bFGF.

Curcumin and demethoxycurcumin are the preferred agents for treating these disorders. The preferred means of administration is to apply the curcumin topically, for example, as an ointment or hydrogel containing between one-half percent (0.5%) and five percent (5%) of the curcumin, or regionally, orally to
5 treat disorders of the gastrointestinal tract or by instillation, to treat bladder or cervical cancer. In alternative embodiments, the curcumin or its analogs can be implanted in the form of one or more pellets of a pharmaceutically acceptable vehicle encapsulating or incorporating the curcumin, or by one or more injections of a pharmaceutically acceptable aqueous solution including the
10 curcumin.

Representative skin disorders include the malignant diseases angiosarcoma, hemangioendothelioma, basal cell carcinoma, squamous cell carcinoma, malignant melanoma and Kaposi's sarcoma, and the non-malignant diseases or conditions including psoriasis, lymphangiogenesis, hemangioma of
15 childhood, Sturge-Weber syndrome, verruca vulgaris, neurofibromatosis, tuberous sclerosis, pyogenic granulomas, recessive dystrophic epidermolysis bullosa, venous ulcers, acne, rosacea, eczema, molluscum contagious, seborrheic keratosis, and actinic keratosis. Representative disorders characterized by increased levels of bFGF include bladder and cervical cancers.

20 As demonstrated in the examples, curcumin and its analogs are potent inhibitors of endothelial cell proliferation, a sensitive test of *in vitro* antiangiogenic effectiveness, and also of corneal neovascularization, a sensitive and reliable test of *in vivo* antiangiogenic effectiveness. The examples demonstrate that this inhibition is exerted directly on the endothelial cells that
25 are primarily involved in angiogenesis, and not indirectly through other effects of these agents. The examples further demonstrate that curcumin and its analogs inhibit the stimulation of angiogenesis *in vivo* by basic fibroblast growth factor.

Brief Description of the Drawings

Figures 1A-C describe the effect of curcumin on endothelial cell
30 proliferation in the absence of basic fibroblast growth factor (bFGF; Figure 1A),

in the presence of bFGF (Figure 1B) and in the absence of bFGF, where the endothelial cells have been transformed (Figure 1C). The figures are graphs of cell number versus concentration of curcumin (μM).

Figures 2A-2B describe the effect of curcumin on the extent of bFGF-stimulated neovascularization in the mouse cornea (Figure 2A), in relation to bFGF-stimulated neovascularization in the absence of curcumin (Figure 2B). The figures are graphs of vessel length (mm) and sector size (clock hours) comparing curcumin ($10 \mu\text{M}$) with control TPCPD, with both in the presence of 80 ng bFGF.

Figures 3A and 3B describe the effect of curcumin and other curcuminoids, tetrahydrocurcumin, bisdemethoxycurcumin, and demethoxycurcumin, on corneal neovascularization, as measured by vessel length (Fig. 3A) and by sector size (Fig. 3B).

Detailed Description of the Invention

I. Disorders to be Treated

Disorders or diseases that can be treated with the angiogenesis inhibitors include those characterized by elevated levels of basic fibroblast growth factor (bFGF), and a number of dermatological disorders.

Diseases and pathological disorders of the skin characterized by angiogenesis in humans include the malignant diseases angiosarcoma, hemangioendothelioma, basal cell carcinoma, squamous cell carcinoma, malignant melanoma and Kaposi's sarcoma, and the non-malignant diseases or conditions psoriasis, lymphangiogenesis, hemangioma of childhood, Sturge-Weber syndrome, verruca vulgaris, neurofibromatosis, tuberous sclerosis, pyogenic granulomas, recessive dystrophic epidermolysis bullosa, venous ulcers, acne, rosacea, eczema, molluscum contagious, seborrheic keratosis, and actinic keratosis.

Examples of disorders characterized by elevated levels of bFGF include bladder cancer (O'Brien, et al. Cancer Res. 57(1):136-140 (1997)) and cervical

cancer (which is caused by a herpes papilloma virus, known to elicit elevated levels of bFGF).

II. Pharmaceutical Compositions

A. Angiogenesis Inhibitors

5 Several different classes of compounds have been determined to be useful as inhibitors of angiogenesis. These include collagenase inhibitors such as metalloproteinases and tetracyclines such as minocycline, naturally occurring peptides such as endostatin and angiostatin, described for example in U.S. patent No. 5,733,876 to O'Reilly, et al., U.S. patent No. 5,290,807, and U.S. Patent No. 10 5,639,725, fungal and bacterial derivatives, such as fumagillin derivatives like TNP-470, the sulfated polysaccharides described in U.S. patent No. 4,900,815 to Tanaka, et al. and the protein-polysaccharides of U.S. patent No. 4,975,422 to Kanoh, et al. and synthetic compounds such as the 2,5-diaryltetrahydrofurans of U.S. patent No. 5,629,340 to Kuwano, et al., aminophenylphosphonic acid 15 compounds of U.S. patent No. 5,670,493 to Cordi, et al., the 3-substituted oxindole derivatives of U.S. patent No. 5,576,330 to Buzzetti, et al., and thalidomides of U.S. patent No. 5,712,291 to D'Amato.

The antibiotics that are useful as angiogenesis inhibitors are those having collagenase inhibitory activity. These include the tetracyclines and chemically 20 modified tetracyclines (CMTs), and three ringed tetracycline homologs, that have the ability to inhibit collagenase but diminished antibacterial activity. Examples of commercially available tetracyclines include chlortetracycline, demeclocycline, doxycycline, lymecycline, methacycline, minocycline, oxytetracycline, rolitetracycline, and tetracycline. The active salts, which are 25 formed through protonation of the dimethylamino group on carbon atom 4, exist as crystalline compounds. These are stabilized in aqueous solution by addition of acid.

Minocycline, a semisynthetic tetracycline antimicrobial, described by Martell, M. J., and Boothe, J. H. in J. Med. Chem., 10: 44-46 (1967), and 30 Zbinovsky, Y., and Chrikian, G. P. Minocycline. In: K. Florey (ed.), Analytical

Profiles of Drug Substances, pp. 323-339 (Academic Press, NY 1977), the teachings of which are incorporated herein, has anticollagenase properties, as reported by Golub, L. M., et al., , J. Periodontal Res., 18: 516-526 (1983); Golub, L. M., et al., J. Periodontal Res. 19: 651-655 (1984); Golub, L. M., et al.,
5 J. Periodontal Res. 20: 12-23 (1985); and Golub, L. M., et al., J. Dent. Res., 66: 1310-1314 (1987). Minocycline, first described in 1967, is derived from the naturally produced parent compounds chlortetracycline and oxytetracycline. The chemically modified tetracyclines are described by U.S. Patent No. 4,704,383 to McNamara, et al., 4,925,833 to McNamara, et al., and 4,935,411 to
10 McNamara, et al., the teachings of which are incorporated herein.

Other exemplary anti-angiogenic compounds include penicillamine and some cytokines such as IL12.

Angiogenesis inhibitors may be divided into at least two classes. The first class, direct angiogenesis inhibitors, includes those agents which are
15 relatively specific for endothelial cells and have little effect on tumor cells. Examples of these include soluble vascular endothelial growth factor (VEGF) receptor antagonists and angiostatin. Basic fibroblast growth factor (bFGF) is a potent, direct angiogenic factor, which has been shown to be a strong stimulus for both endothelial proliferation and migration, *in vitro* and *in vivo*. The
20 activity of bFGF on endothelial cells may be due in part to stimulation of protein kinase C. Shing et al., Science 223:1296-1299 (1984); Kent et al., Circ. Res. 77:231-238 (1995). Blockage of bFGF's stimulation of endothelial cells can inhibit angiogenesis.

Indirect inhibitors may not have direct effects on endothelial cells but
25 may down-regulate the production of an angiogenesis stimulator, such as VEGF. Arbiser et al., Molec. Med. 4:376-383 (1998). VEGF has been shown to be up-regulated during chemically induced skin carcinogenesis; this is likely due to activation of oncogenes, such as H-ras. Arbiser et al., Proc. Natl. Acad. Sci. U. S. A. 94:861-866 (1997); Larcher et al., Cancer Res. 56:5391-5396 (1996); Kohl
30 et al., Nature Med. 1:792-797 (1995). Examples of indirect inhibitors of

angiogenesis include inhibitors of ras-mediated signal transduction, such as farnesyltransferase inhibitors.

Direct inhibition of endothelial cell proliferation can be assayed in cell culture systems, in which the effects of specific factors which control the complex process of angiogenesis can be studied. Effects discovered in such *in vitro* systems can then be studied in *in vivo* systems. Kenyon et al., Invest. Ophthalmol. 37:1625-1632 (1996).

Curcumin (diferuloylmethane) and certain of its analogs, together termed curcuminoids, are well known natural products, recognized as safe for ingestion by and administration to mammals including humans. Bille et al., Food Chem. Toxicol. 23:967-971 (1985). Curcumin is a yellow pigment found in the rhizome of *Curcuma longa*, the source of the spice turmeric. Turmeric has been a major component of the diet of the Indian subcontinent for several hundred years, and the average daily consumption of curcumin has been found to range up to 0.6 grams for some individuals, without reported adverse effects. Food-grade curcumin consists of the three curcuminoids in the relative amounts: 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin. Thimmayamma et al., Indian J. Nutr Diet 20:153-162 (1983); Bille et al., Food Chem. Toxicol. 23:967-971 (1985). The fully saturated derivative tetrahydrocurcumin is also included in the term *curcuminoid*.

Curcumin can be obtained from many sources, including for example Sigma-Aldrich, Inc. The curcumin analogs demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin can also be obtained from many sources, or readily prepared from curcumin by those skilled in the art.

Curcumin has been used in indigenous Indian medicine for several hundred years, as a topical agent for sprains and inflammatory conditions, in addition to oral use to promote health and treat digestive and other disorders. Absorption of ingested or orally administered curcumin is known to be limited, and absorbed curcumin is rapidly metabolized. Govindarajan, CRC Critical

Rev. Food Sci Nutr. 12:199-301 (1980); Rao et al., Indian J. Med. Res. 75:574-578 (1982).

Numerous effects of the ingestion or oral administration of the curcuminoids have been reported, based on controlled research, population studies, case reports and anecdotal information. Evidence of chemopreventive activity of curcumin administered orally has led to clinical trials sponsored by the National Cancer Institute, regarding prevention of cancer. Kelloff et al., J. Cell. Biochem. Suppl. 26:1-28 (1996). Oral administration of curcumin to mice treated with skin and colon chemical carcinogens has been shown to result in a decreased incidence and size of induced tumors compared with control mice. Huang, et al., Cancer Res. 54:5841-5847 (1994); Huang et al., Carcinogenesis 16:2493-2497 (1995); Huang et al., Cancer Lett. 64:117-121; Rao et al., Cancer Res. 55:259-266 (1995); Conney et al., Adv Enzyme Regul. 31: 385-396 (1991).

Huang, et al. found that the oral administration of three curcuminoid compounds curcumin, demethoxycurcumin and bisdemethoxycurcumin were able to inhibit phorbol ester-stimulated induction of ornithine decarboxylase and promotion of mouse skin initiated with 7,12-dimethylbenzanthracene (DMBA). These compounds also inhibited phorbol ester-mediated transformation of JB6 cells. The saturated derivative tetrahydrocurcumin was less active than the unsaturated analogs in these assays. Huang et al., Carcinogenesis 16:2493-2497 (1995).

The mechanism or mechanisms of curcumin's chemopreventive activities were not previously understood, although it was recognized as an antioxidant and was known to exhibit antimutagenic activity in the Ames Salmonella test and to produce biochemical effects similar to those of the polyphenols, chemopreventive agents found in green tea. Stoner, J. Cell. Biochem. Suppl. 22:169-180 (1995). Curcumin has been demonstrated to inhibit several signal transduction pathways, including those involving protein kinase, the transcription factor NF-kB, phospholipase A2 bioactivity, arachidonic acid metabolism, antioxidant activity, and epidermal growth factor (EGF) receptor

autophosphorylation. Lu et al., Carcinogenesis 15:2363-2370 (1994); Singh et al., J. Biol. Chem. 270:24995-25000 (1995); Huang et al., Proc. Natl. Acad. Sci. U. S. A. 88:5292-5296 (1991); Korutla et al., Carcinogenesis 16:1741-1745 (1995); Rao et al., Carcinogenesis 14:2219-2225 (1993).

- 5 Because of the complexity of the factors that regulate or effect angiogenesis, and their specific variation between tissues and according to circumstances, the response to a specific agent may be different or opposite, in different tissues, under different physiological or pathological conditions and between *in vitro* and *in vivo* conditions. For example, U. S. Patent No.
- 10 5,401,504 to Das et al., discloses that oral or topical administration of turmeric to animals including humans promotes wound healing, and postulates that it acts in part through stimulation of angiogenesis, although this postulate was not experimentally verified. Administration of curcumin has been reported to inhibit smooth muscle cell proliferation *in vitro*. Huang, et al., European J.
- 15 Pharmac. 221:381-384 (1992). U. S. Patent No. 5,891,924 to Aggarwal discloses that oral administration of curcumin to animals inhibits activation of the transcription factor NF-kB, and claims its use in pathophysiological states, particularly specific conditions involving the immune system. Several biochemical actions of curcumin were studied in detail, but no single action was
- 20 reported to be responsible for these effects of curcumin. Singh et al. reported that curcumin inhibits *in vitro* proliferation of human umbilical vein endothelial cells (HUVEC) and suggested that it might have anti-angiogenic activity. However, this inhibition was independent of basic fibroblast growth factor stimulation of the proliferation of endothelial cells, and *in vivo* studies were not
- 25 reported. Singh et al., Cancer Lett. 107:109-115 (1996). Thaloor et al. disclosed inhibition by curcumin of HUVEC growth and formation of tube structures on Matrigel, in a model of capillary formation, and ascribed this inhibition to modulation of metalloproteinases of the HUVEC. Thaloor et al., Cell Growth Differ. 9:305-312 (1998).

As demonstrated by the examples, these are not the mechanism involved in inhibition of angiogenesis as described herein, and accordingly, the disorder to be treated and the dosage and means of administration are different, based on the role of curcuminoids in inhibiting bFGF.

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
2210
2211
2212
2213
2214
2215
2216
2217
2218
2219
2220
2221
2222
2223
2224
2225
2226
2227
2228
2229
2230
2231
2232
2233
2234
2235

B. Carriers

Pharmaceutical compositions containing the angiogenesis inhibitor are prepared based on the specific application. Application can be either topical, localized, or systemic. Any of these compositions may also include

5 preservatives, antioxidants, antibiotics, immunosuppressants, and other biologically or pharmaceutically effective agents which do not exert a detrimental effect on the normal tissue to be treated.

Compositions for local or systemic administration will generally include an inert diluent. Solutions or suspensions used for parenteral, intradermal,

10 subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as

15 ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Systemic Carriers

20 Inhibitors can be systemically administered either parenterally or enterally. The composition can be administered by means of an infusion pump, for example, of the type used for delivering insulin or chemotherapy to specific organs or tumors, by injection, or by depo using a controlled or sustained release formulation. In a preferred systemic embodiment, drugs are administered orally,

25 in an enteric carrier if necessary to protect the drug during passage through the stomach.

The angiogenic inhibitors can be administered systemically by injection in a carrier such as saline or phosphate buffered saline (PBS) or orally, in the case of an inhibitor such as thalidomide, in tablet or capsule form. The tablets or

30 capsules can contain any of the following ingredients, or compounds of a similar

nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; or a glidant such as colloidal silicon dioxide. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

Local or Topical Carriers

The angiogenic inhibitors can also be applied locally or topically, in a carrier such as saline or PBS, in an ointment or gel, in a transdermal patch or bandage, or controlled or sustained release formulation. Local administration can be by injection at the site of the injury, or by spraying topically onto the injury. The inhibitor can be absorbed into a bandage for direct application to the wound, or released from sutures or staples at the site. Incorporation of compounds into controlled or sustained release formulations is well known.

For topical application, the angiogenesis inhibitor is combined with a carrier so that an effective dosage is delivered, based on the desired activity, at the site of application. The topical composition can be applied to the skin for treatment of diseases such as psoriasis. The carrier may be in the form of an ointment, cream, gel, paste, foam, aerosol, suppository, pad or gelled stick. A topical composition for use of an ointment or gel consists of an effective amount of angiogenesis inhibitor in an ophthalmically acceptable excipient such as buffered saline, mineral oil, vegetable oils such as corn or arachis oil, petroleum jelly, Miglyol 182, alcohol solutions, or liposomes or liposome-like products.

In a preferred form for controlled release, the composition is administered in combination with a biocompatible polymeric implant which releases the angiogenesis inhibitor over a controlled period of time at a selected site. Examples of preferred biodegradable polymeric materials include polyanhydrides, polyorthoesters, polyglycolic acid, polylactic acid, polyethylene

vinyl acetate, and copolymers and blends thereof. Examples of preferred non-biodegradable polymeric materials include ethylene vinyl acetate copolymers. These can be prepared using standard techniques as microspheres, microcapsules, tablets, disks, sheets, and fibers.

- 5 An implantable pellet is the preferred mode of local administration of these agents to tissues. The preferred concentration of curcuminoid agent delivered locally to the target tissue is greater than 10 micromolar, preferably 10-50 micromolar.

III. Methods for Treatment

- 10 For the treatment of skin disorders, the angiogenesis inhibitors are administered topically or regionally. In a preferred embodiment, the inhibitors are administered in an ointment, salve or other pharmaceutically acceptable carrier. For treatment of certain disorders characterized by elevated levels of bFGF, the angiogenesis inhibitors, preferably curcumin or demethoxycurcumin
15 or another curcuminoid compound, or a combination of two or more curcuminoid compounds, is applied topically in diseases or pathologic conditions of the skin, or locally in other tissues, to treat cancer, pre-malignant conditions and other diseases and conditions in which angiogenesis occurs. The preferred means of administration is to apply the curcumin topically, for
20 example, as an ointment or hydrogel containing between one-half percent (0.5%) and five percent (5%) of the curcumin, or regionally, orally to treat disorders of the gastrointestinal tract or by instillation, to treat bladder or cervical cancer.

- The administration of these agents topically or locally may also used to prevent initiation or progression of such diseases and conditions. For example, a
25 curcuminoid formulation may be administered topically or by instillation into a bladder if a biopsy indicated a pre-cancerous condition or into the cervix if a Pap smear was abnormal or suspicious.

- The angiogenesis inhibiting formulation is administered as required to alleviate the symptoms of the disorder. Assays can be performed to determine an
30 effective amount of the agent, either *in vitro* and *in vivo*. Representative assays

are described in the examples provided below. Other methods are known to those skilled in the art, and can be used to determine an effective dose of these and other agents for the treatment and prevention of diseases or other disorders as described herein.

5 The present invention will be further understood by reference to the following non-limiting examples.

As demonstrated in the examples, curcumin inhibits basic fibroblast growth factor (bFGF)-induced proliferation of endothelial cells *in vitro* and angiogenesis *in vivo*. The effect of curcumin and curcumin analogs with known
10 differential chemopreventive activities, demethoxycurcumin, tetrahydrocurcumin, and bisdemethoxycurcumin, on *in vivo* angiogenesis was also demonstrated. Curcumin had a strong antiproliferative effect on endothelial cells, with a steep curve occurring between 5 and 10 μ M. This was true both in the presence or absence of bFGF, and this inhibition could not be overcome by
15 the immortalizing ability of SV40 large T antigen. The corneal neovascularization assay, which measures increased vessel length and density *in vivo*, in response to a bFGF pellet placed in the normally avascular cornea, has proven useful in the confirmation and characterization of multiple angiogenesis inhibitors. The inhibition of bFGF-mediated corneal neovascularization by
20 curcumin and its derivatives is evidence that curcumin is a direct angiogenesis inhibitor *in vivo*. This inhibition was not due to dilution of bFGF, as administration of a structurally related inactive compound, tetraphenylcyclopentadienone (TPCPD), had no effect on bFGF-induced corneal neovascularization. The lack of inhibition of TPA-mediated VEGF production
25 further supports the role of curcumin as a direct angiogenesis inhibitor.

The following materials and methods were used in the examples.

MATERIALS AND METHODS

Endothelial Proliferation Assays

Bovine capillary endothelial cells were isolated according to the method
30 of Folkman, et al., Proc. Nat. Acad. Sci. U. S. A. 76:5217-5221 (1979), and were

plated at a concentration of 10,000 cells/well in gelatinized 24-well dishes. The primary endothelial cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine serum and grown at 37°C in 10% CO₂. Twenty-four hours after plating, cells were treated with curcumin in the presence or absence of bFGF. After 72 hours of treatment, cells were counted using a Coulter counter. Cell counts for each condition were repeated in triplicate and in the presence or absence of 1ng/ml bFGF.

Similarly, MSI (ATCC CCRL 2279) endothelial cells, which are a SV40 large T antigen immortalized murine endothelial cell line, were also plated at a concentration of 10,000 cells/well in nongelatinized 24-well dishes. MSI cells do not require endothelial mitogens for growth and were cultured in DMEM supplemented with 5% fetal calf serum (FCS). Cells were counted after a 72-hour exposure to curcumin with the same method used for the bovine capillary endothelial cells.

Corneal Neovascularization

C57BL6 male mice (5-6 weeks old) were anesthetized with methoxyflurane prior to implantation of pellets and with 0.5% proparacaine. A central, intrastromal linear keratotomy was performed with a surgical blade, and a lamellar micropocket was prepared according to the method of Kenyon, et al. (1996). The pellet was advanced to the end of the pocket. Erythromycin ointment was placed on the operated eye to prevent infection. Eyes were examined by slit lamp on days 3-6 after implantation under general anesthesia. Corneal angiogenesis was assayed through two measurements.

Vessel length is the length of the vessel from the corneal limbus as it grows toward the pellet containing bFGF.

Sector size is a measurement of neovascularized area of the cornea. The cornea is viewed as a circle that can be divided into twelve sectors of 30 degrees span each, analogous to the division of a clock face into twelve hours. Thus, neovascularization of a sector corresponding to one fourth of the cornea would be recorded as a sector size measurement of three. This system of measurement,

recording sector sizes as “clock hours”, was established by Kenyon et al., Invest. Ophthalmol. 37:1625-1632 (1996).

Production of VEGF mRNA in HaCaT Keratinocytes

HaCaT keratinocytes were grown in (DMEM) (JRH) supplemented with
5 5% FCS (Hyclone, Logan, UT) in 25cm² flasks. One hour prior to stimulation
with 12-O-tetradecanoylphorbol-13-acetate (TPA), cells were switched to
serumless media supplemented with 10µM curcumin or an equal quantity of
ethanol (final concentration 0.1%). TPA was added to a final concentration of
5ng/ml and cells were incubated for three hours at 37°C and harvested, and their
10 RNA was extracted with guanidinium thiocyanate/phenol.

Phase II Enzyme Induction

The ability of curcumin derivatives to induce phase II activities was
measured by assaying quinone reductase [NAD(P)H:(quinone-acceptor)
oxidoreductase, EC1.6.99.2] in murine Hepac1c7 cells. Serial dilutions of
15 curcumin, curcumin derivatives, and tetraphenylcyclopentadienone (TPCPD)
were added, and the concentration of compound required to double the specific
activity (CD) was calculated according to the method of Prochaska, et al., Proc.
Natl. Acad. Sci. U. S. A. 89:2394-2398 (1992).

Materials

20 Curcumin, TPA and TPCPD were obtained from Sigma-Aldrich, Inc.
Curcumin analogs (bisdemethoxycurcumin, demethoxycurcumin and
tetrahydrocurcumin) were provided by Dr. A. R. Conney of Rutgers-The State
University of New Jersey.

C57BL6 mice were obtained from Charles River Laboratories. The MS1
25 transformed cells were developed by Dr. J. L. Arbiser and deposited with the
ATCC (ATCC CCRI 2279).

Implant Pellets

Pellets were prepared according to a modification of the method of
Kenyon, et al. Invest. Ophthalmol. Vis. Sci. 37:1625-1632 (1996). An aqueous
30 solution of 18 mcg of basic fibroblast growth factor (Scios Nova, Mountain

View, California) was evaporated to dryness under reduced pressure in the presence of 10 mg of sucralfate (Bukh Meditec, Vaerloose, Denmark) Ten microliters of 12% hydron and 10 mg of curcumin or curcumin analog were then added, and the homogenous mixture was deposited onto a sterile 15 x 15 mm 3-
5 300/50 Nylon mesh (Tetko, Lancaster, NY) and air dried. Once the mixture was dry, the mesh was manually dissociated to yield 225 pellets. Each pellet contained 80 ng of bFGF and 44 µg of curcumin or curcumin analog. Pellets containing hydron in the absence of bFGF do not cause neovascularization, so pellets prepared in the absence of bFGF were not used in this study. The
10 approximate pore size was 0.4 x 0.4mm. Both sides of the mesh were covered with a thin layer of hydron.

Isotopically Labelled Antisense Riboprobe

A plasmid containing the coding region of human vascular endothelial growth factor (VEGF) 121 was obtained from H. Welch (University of Freiburg,
15 Germany), and used to generate P³²-labeled antisense riboprobe as per manufacturers protocols (Ambion, Austin, TX). RNase protection assays were performed according to the method of Hod, Biotechniques 13:852-853 (1992). Protected fragments were separated on gels of 5% acrylamide, 8 M urea, 1 x Tris-borate buffer, and quantified with a phosphorimager (Molecular Dynamics,
20 Sunnyvale, CA). An 18S riboprobe was included in each sample to normalize for variations in loading and recovery of RNA.

Measurement and Analysis

Significant differences between two groups were determined using an unpaired, two-tailed Student's t-test. Results are expressed as the mean plus or
25 minus the standard error of the mean.

Example 1: Curcumin inhibition of endothelial cell proliferation is dependent on curcumin dose and the presence or absence of basic fibroblast growth.

Endothelial cells were stimulated to proliferate in the presence of 1ng/ml
5 bFGF. Curcumin was added in concentrations ranging from 0.5 to 10 μ M to primary endothelial cells.

Figures 1A-C describe the effect of curcumin on endothelial cell proliferation in the absence of basic fibroblast growth factor (bFGF; Figure 1A), in the presence of bFGF (Figure 1B) and in the absence of bFGF, where the
10 endothelial cells have been transformed (Figure 1C). A steep decrease in cell number was seen at 10 μ M. No evidence of cytotoxicity was observed, and the number of cells at the end of treatment was not significantly less than the number cells originally plated. This decrease in proliferation due to curcumin concentration of 10 μ M was observed in both the presence or absence of bFGF.

15 In addition, curcumin was able to inhibit the growth of endothelial cells immortalized by SV40 large T antigen, with a similar dose response as seen with primary endothelial cells.

Example 2: Curcumin inhibition of corneal neovascularization is dependent on the presence of basic fibroblast growth factor.

20 The ability of curcumin to inhibit bFGF-induced corneal neovascularization *in vivo* was measured. Pellets were prepared containing 80 ng of bFGF and curcumin, or a control aromatic ketone, tetraphenylcyclopentadienone (TPCPD). TPCPD was added to rule out the possibility that the inhibition of neovascularization due to curcumin was not
25 secondary to dilution. Neovascularization was assessed by slit lamp at 5 days after implantation, and the corneas were photographed.

Figures 2A-2B describe the effect of curcumin on the extent of bFGF-stimulated neovascularization in the mouse cornea (Figure 2A), in relation to bFGF-stimulated neovascularization in the absence of curcumin (Figure 2B).
30 There was no difference in neovascularization in mice containing bFGF pellets

in the presence or absence of TPCPD. Both the vessel length and sector sizes were significantly reduced in the presence of curcumin.

Example 3: Curcumin and other curcumin analog inhibition of corneal neovascularization in the presence of basic fibroblast growth factor is dependent on the dose and structure of the curcuminoid.

Three curcumin analogs were assayed for their ability to inhibit bFGF-induced corneal neovascularization as described above.

Figures 3A and 3B describe the effect of curcumin and other curcuminoids, tetrahydrocurcumin, bisdemethoxycurcumin, and demethoxycurcumin, on corneal neovascularization, as measured by vessel length (Fig. 3A) and by sector size (Fig. 3B). All analogs showed inhibitory activity, with demethoxycurcumin showing the greatest activity on both sector size and vessel length, tetrahydrocurcumin having the least effect on sector size, and bisdemethoxycurcumin having the least effect on vessel length. All of the curcumin analogs showed significant inhibition of bFGF-mediated neovascularization compared with control pellets.

Example 4: Curcumin does not inhibit vascular endothelial growth factor mRNA production in transformed keratinocytes.

HaCaT cells are derived from spontaneously transformed human keratinocytes. In order to determine whether curcumin could inhibit production of angiogenesis factors by relevant tumor cells as well as directly inhibit endothelial function; HaCaT cells were treated with tetradecanoylphorbol ester (TPA) in the presence or absence of curcumin and expression of VEGF mRNA was measured.

TPA caused a 2.5-fold increase in VEGF mRNA. This increase was not inhibited by curcumin. Thus the primary antiangiogenic effect of curcumin is directly on endothelium, rather than inhibition of production of VEGF, an important angiogenic factor.

Example 5: Inhibition of corneal neovascularization by curcumin and other curcuminoids does not correlate with the induction of Phase II enzymes by curcumin and other curcuminoids.

Several plant-derived compounds with anticancer and chemopreventive activities also show the ability to induce phase II detoxifying enzymes, including quinone reductase. To determine whether the antiangiogenic activities of curcumin derivatives correlated with the ability to induce quinone reductase activity, the concentration needed to double the specific activity value (CD) was determined.

All of the curcumin analogs studied except tetrahydrocurcumin had approximately equal potencies in induction of phase II enzymes, a measure of detoxification activity, whereas the fully saturated tetrahydrocurcumin has little ability to induce phase II enzymes. Tetrahydrocurcumin, the curcumin derivative with the least antitumor activity, caused a 1.6-fold induction of quinone reductase activity at the highest concentration tested, 25 μ M. However, TPCPD, which is an unsaturated aromatic ketone with no anti-angiogenic activity, had a CD value of 4.8 μ M. The results are shown in Table 1. Thus, antiangiogenic activity does not correlate with phase II activity. This finding is evidence that the two processes are not based on similar mechanisms.

Modifications and variations of the methods and compositions described herein will be obvious to those skilled in the art and are intended to come within the scope of the appended claims.

**Table 1: Actual and Relative Effects of Curcuminoids and TPCPD
On Phase II Enzyme Induction and Angiogenesis**

PHASE II

<u>COMPOUND</u>	<u>INDUCTION</u>		<u>ANTI-ANGIOGENIC EFFECT</u>			
	CD ¹	Rank ²	Sector Size ³ (μ M)	Rank ²	Vessel Length (mm.)	Rank ²
Tetrahydro- curcumin	>25	1	2.43	2	0.74	3
Bisdemethoxy- curcumin	11.0	2	1.7	3	0.88	2
Demethoxy- curcumin	9.0	3	0.71	5	0.26	5
Curcumin	7.3	4	1.17	4	0.59	4
TPCPD	4.8	5	3.72	1	1.16	1

Notes:

1. Concentration to double the measured specific activity; negatively correlated with effectiveness
2. Rank: Relative effectiveness in Phase II enzyme induction or in antiangiogenic effect (reduction of sector size or vessel length)
3. Sector size expressed in units of 1/12 of a circle, or 30 degrees (equivalent to "clock hours")